



**Syddansk Universitet**

## **Early life mortality risks in opposite-sex and same-sex twins a Danish cohort study of the twin testosterone transfer hypothesis**

Juel Ahrenfeldt, Linda ; Larsen, Lisbeth Aagaard; Lindahl-Jacobsen, Rune; Skytthe, Axel; Hjelmberg, Jacob v. B.; Möller, Sören; Christensen, Kaare

*Published in:*  
Annals of Epidemiology

*DOI:*  
[10.1016/j.annepidem.2016.11.011](https://doi.org/10.1016/j.annepidem.2016.11.011)

*Publication date:*  
2017

*Document version*  
Peer reviewed version

*Document license*  
CC BY-NC-ND

*Citation for pulished version (APA):*  
Juel Ahrenfeldt, L., Larsen, L. A., Lindahl-Jacobsen, R., Skytthe, A., Hjelmberg, J. V. B., Möller, S., & Christensen, K. (2017). Early life mortality risks in opposite-sex and same-sex twins: a Danish cohort study of the twin testosterone transfer hypothesis. *Annals of Epidemiology*, 27(2), 115–120.e2. DOI: [10.1016/j.annepidem.2016.11.011](https://doi.org/10.1016/j.annepidem.2016.11.011)

### **General rights**

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal ?

### **Take down policy**

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.



Published in final edited form as:

*Ann Epidemiol.* 2017 February ; 27(2): 115–120.e2. doi:10.1016/j.annepidem.2016.11.011.

## Early-life mortality risks in opposite-sex and same-sex twins: a Danish cohort study of the twin testosterone transfer hypothesis

Linda Juel Ahrenfeldt, PhD<sup>a,b,\*</sup>, Lisbeth Aagaard Larsen<sup>a</sup>, Rune Lindahl-Jacobsen, PhD<sup>a,b</sup>, Axel Skytthe, PhD<sup>a</sup>, Jacob v.B. Hjelmberg, PhD<sup>a</sup>, Sören Möller, PhD<sup>a</sup>, and Kaare Christensen, MD, PhD<sup>a,c,d</sup>

<sup>a</sup>Department of Public Health, The Danish Twin Registry, Unit of Epidemiology, Biostatistics and Biodemography, University of Southern Denmark, Odense C, Denmark

<sup>b</sup>Department of Public Health, Max-Planck Odense Center on the Biodemography of Aging, University of Southern Denmark, Odense C, Denmark

<sup>c</sup>Department of Clinical Biochemistry and Pharmacology, Odense University Hospital, Odense C, Denmark

<sup>d</sup>Department of Clinical Genetics, Odense University Hospital, Odense C, Denmark

### Abstract

**Purpose**—To investigate the twin testosterone transfer (TTT) hypothesis by comparing early-life mortality risks of opposite-sex (OS) and same-sex (SS) twins during the first 15 years of life.

**Methods**—We performed a population-based cohort study to compare mortality in OS and SS twins. We included 68,629 live-born Danish twins from 1973 to 2009 identified through the Danish Twin Registry and performed piecewise stratified Cox regression and log-binomial regression.

**Results**—Among 1933 deaths, we found significantly higher mortality for twin boys than for twin girls. For both sexes, OS twins had lower mortality than SS twins; the difference persisted for the first year of life for boys and for the first week of life for girls.

**Conclusions**—Although the mortality risk for OS boys was in the expected direction according to the TTT hypothesis, the results for OS girls pointed in the opposite direction, providing no clear evidence for the TTT hypothesis.

### Keywords

Mortality; Twins; Opposite-sex; Same-sex; Sex

\*Corresponding author. The Danish Twin Registry, Unit of Epidemiology, Biostatistics and Biodemography, University of Southern Denmark, 5000 Odense C, Denmark. Tel.: +45 65503844; fax: +45 25365927. lahrenfeldt@health.sdu.dk (L.J. Ahrenfeldt).

The authors have no competing interests to declare.

## Introduction

It is well known that boys have higher risk of infant mortality and morbidity than girls [1,2]. Sex differences in mortality have been explained by a combination of biological, social, and environmental factors, but lifestyle and behavioral factors are less likely to explain sex difference in infant mortality [3]. Studies of animal models find that males exhibit reduced immune responses and increased intensity and prevalence of infections compared with females [4]. These sex differences may reflect the immunosuppressive effects of testosterone, as well as the positive effects of progesterone and estradiol on immune responses [4,5]. Whether these findings apply to humans is unknown [1], but there is evidence that girl infants have lower mortality from infections [6] and respiratory conditions [7] compared with boys. It is hypothesized that the male disadvantage begins *in utero* [8], where the gonadal steroid production differs between the sexes [3].

Twin studies offer unique opportunities to investigate mechanisms underlying sex differences. There is evidence suggesting that male sex hormones could influence the female fetus in opposite-sex (OS) twins. For instance, studies in mammals have found that female fetuses positioned between two males are likely to express masculinization of anatomical, physiological, and behavioral traits in adult life [9]. Sex hormones are lipid soluble steroids capable of crossing fetal membranes [10]. The twin testosterone transfer (TTT) hypothesis states that human fetuses gestated with a male co-twin are masculinized in development, which may be due to the exposure of prenatal androgens [11]; however, the evidence remains inconclusive (for reviews, see [11,12]).

Hence, early-life mortality risks in OS and same-sex (SS) twins may also differ due to zygosity differences. OS twins are always dizygotic (DZ), whereas SS twins are either monozygotic (MZ) or DZ. A large part of MZ twins (70%–75%) are monochorionic (MC), sharing only one placenta, and the remaining part has completely separate placentas and membranes (dichorionic [DC]) [13]. MC twins are at increased risk for perinatal mortality and morbidity compared with DC twins [14].

The literature comparing OS and SS twins with regard to early-life mortality risks is limited and inconsistent, but higher infant mortality for SS boys than for OS boys [15–17] and adverse outcomes including increased respiratory morbidity and higher mortality for OS twin girls in agreement with the TTT hypothesis have been reported [18–20]. Our study using data on a sample of 68,629 Danish twins aims to compare mortality in twin boys and girls and in OS and SS twins by age and over time within the first 15 years of life.

## Materials and methods

### Material

This study consists of all twins born in Denmark during the period 1973–2009. The twins were identified through the Danish Twin Registry [21,22]. The Danish Twin Registry has collected data on twins including zygosity of SS twin pairs born up to 2000, which is based on questions about the degree of co-twin similarity [23]. However, both twins have to be alive beyond infancy to have their zygosity assessed by questionnaire and therefore zygosity

is generally available only for twin pairs surviving infancy [21]. All twins both live-born and stillborn have been identified through the Danish Medical Birth Registry since 1973 [24]. For these twins, information about the personal identification numbers for the infant and the mother, information on deaths among live-born twins, as well as information on emigrations came from the Danish Civil Registration System [25]. Information on causes of death was obtained from the Danish Registry of Causes of Death [26]. Information on maternal education came from the Danish Education Registers [27], and information about birth weight was obtained from the Medical Birth Registry [28]. In total, 68,629 live-born twins were eligible for analyses (Table 1). For the birth cohorts 1973–1996, information about stillborn twins was available for 34,736 twins.

## Variables

Possible confounders in this study were year of birth, maternal education, and maternal age. Year of birth ranged from 1973 to 2009 and was stratified into decades (birth cohorts: 1973–1979, 1980–1989, 1990–1999, 2000–2009). Maternal education refers to the highest completed education until year 2012. The categorization of education was based on the International Standard Classification of Education [29]. Three groups were distinguished: primary and lower secondary (<10 years), upper and postsecondary (10–12 years), and tertiary (>12 years). Maternal age at delivery was categorized as younger than 20 years, 20–34 years, and 35 years and older. Birth weight was considered an intermediate factor in the association between OS/SS twins and mortality. However, all analyses were repeated including birth weight, and the results were similar (not shown). Based on age-at-death, we generated four separate risk periods according to the definitions of the categories of infant deaths: early neonatal deaths (0–7 days), late neonatal deaths (8–28 days), postneonatal deaths (29–365 days), and child mortality (1–15 years) [30].

## Statistical analyses

Analyses of differences in categorical baseline characteristics (emigration and maternal education) for OS and SS twins were performed by  $\chi^2$  tests. Differences in continuous background variables (maternal age and birth weight) were investigated using  $t$  tests. Relative risks (RRs) for child mortality (0–15 years) for each of the possible confounders were calculated stratified by sex applying log-binomial regression both crude and adjusted for the other covariate. All estimates were adjusted for decades.

To analyze the associations of mortality between girls and boys and between OS and SS twins, we used piecewise stratified Cox regression, adjusting for the nonindependence of twins in a pair [31]. The twins were followed up for 15 years or until July 1, 2013, whichever came first. All associations were expressed as hazard ratios (HRs) with 95% confidence intervals (CIs). The Cox proportional hazards assumption was fulfilled in all risk periods. Mortality HRs, taking into account emigrations as censoring, were reported including adjustment for decades, crude, and adjusted for maternal age and education. Analyses of sex differences were done within all twins as well as within intact OS twin pairs. All analyses were repeated within each decade, and analyses of OS and SS twins were conducted separately for each sex. Cause-specific mortality was classified according to the main groups in the International Classification of Disease 8 and 10 (9 was never used in

Denmark). The associations of OS/SS twins with cause-specific mortality were investigated using  $\chi^2$  test.

We performed two sensitivity analyses regarding zygosity: First, we included all twins with unknown zygosity (UZ) in the MZ and same-sex dizygotic (ssDZ) twin groups, respectively, and compared these twin groups with the OS twins. Second, the UZ twins were randomly distributed (50:50) between the MZ and ssDZ twins. These groups were then compared with the OS twins and this analysis was repeated 10 times with this random distribution of the UZ twins. Moreover, differences in the risk of stillbirth and perinatal mortality between girls and boys and between OS and SS twins born between 1973 and 1996 were investigated. This was done using log-binomial regression estimating RRs and 95% CIs. A stillbirth was defined as a delivery of a dead fetus at 28 or more completed weeks of gestation. Perinatal mortality was defined as stillbirths and deaths of live-born infants within the first seven days of life.

We estimated the overall mortality (0–15 years) for each decade for live-born boy and girl twins using Kaplan-Meier estimation, which were performed stratified by OS/SS twin status. In addition, we estimated HRs and 95% CIs for the differences in mortality between the different decades through a stratified Cox proportional hazards analysis for OS and SS twins as well as for all twins.

## Results

A total of 1933 deaths of all causes were observed in 68,629 twins (Table 1). Differences in neonatal deaths (early and late), postneonatal deaths and child mortality for live-born boys and girls, and OS and SS twins are shown in Table 2. We found that boys had significantly higher mortality than girls at all ages. For stillbirth and perinatal mortality, the significant sex differences were verified (Table 3). When we compared the mortality for boys and girls within OS twin pairs, the significant differences vanished in all age groups.

Among live births, OS twins had significantly lower mortality than SS twins for both sexes. For boys, a significantly lower mortality for OS than SS twins was found for both early neonatal deaths (HR: 0.66; 95% CI: 0.55–0.79), late neonatal deaths (HR: 0.62; 95% CI: 0.40–0.96), and postneonatal deaths (HR: 0.63; 95% CI: 0.43–0.92). For girls, the difference was only found for early neonatal deaths (HR: 0.74; 95% CI: 0.61–0.90) (Table 2 and Fig. 1). Adjustments for maternal age and education did not change the effect estimates to any substantial degree (Table 2).

Of the six groups of death causes (Supplementary Table 1), we did not find significant differences between OS and SS twins except from higher risk of external causes (injuries) for OS compared with SS girls (3.6% vs. 0.9%,  $P = .007$ ). However, when comparing OS and SS twins regarding infectious and respiratory diseases separately, we found that OS boys had lower mortality from infectious diseases compared with SS boys (0.4% vs. 2.5%,  $P = .032$ ) (results not shown).

Both sensitivity analyses confirmed a lower mortality for OS compared with ssDZ twins. All HRs were below 1 for both sexes. For boys, all associations were significant in the neonatal

period and the HRs ranged between 0.61 and 0.73. For girls, the HRs ranged between 0.74 and 0.87, but only few associations were significant (results not shown).

The analyses of stillbirths confirmed a lower mortality for OS than for SS twins of both sexes, RR: 0.48 (95% CI: 0.36–0.65) for boys and 0.42 (95% CI: 0.29–0.59) for girls. The same pattern was found when analyzing perinatal mortality (Table 3).

Overall mortality within the first 15 years of life has decreased during the last 4 decades in Denmark for OS and SS twins of both sexes. For live-born boys, the mortality decreased from 5.0% to 1.9% for OS and from 7.2% to 2.3% for SS twins. For girls, the mortality decreased from 4.0% to 1.7% for OS and from 4.6% to 1.9% for SS twins (Figs. 2 and 3). From the 1990s to the 2000s, the decrease in mortality was only significant for SS boys, and for OS boys, a tendency was found in the opposite direction toward higher mortality with time (HR: 1.23; 95% CI: 0.90–1.69) (Supplementary Table 2).

The raw associations between the potential confounding variables and mortality showed an inverse association between maternal education and child mortality for both sexes, however, only significant for twin girls whose mothers had the lowest education. Maternal age was not significantly associated with mortality for twins; however, a tendency was indicated toward higher mortality among twins with mothers below age 20 and a lower mortality for twins with mothers above age 35 (Supplementary Table 3).

## Discussion

A large population-based cohort study was used to compare mortality in OS and SS twins within the first 15 years of life. We confirmed the known sex differences in mortality. For live-born boys, the results demonstrated a significantly lower mortality for OS than for SS twins in the neonatal (early and late) and postneonatal periods. There are at least two possible explanations for this: first, the inclusion of the MZ twins in the SS twin group; second, lower exposure to testosterone in *utero* for OS than for SS boys. For girls, we found similar mortality between live-born OS and SS twins, except that OS girls had the lowest mortality within the early neonatal period. The risk of stillbirth was significantly lower for OS than for SS twins for both sexes. An overall decrease in mortality was observed for both OS and SS twins during the last 4 decades in Denmark.

The present study agreed with previous twin studies reporting higher mortality for male-male than for female-female twins [15–17]. However, we found that within OS twin pairs, sex-related differences in mortality were much less pronounced with no significant mortality differences between girls and boys, also consistent with earlier reports [17,32].

The lower perinatal mortality for OS than for SS twins may at least partly be due to the MZ twins being in the SS twin groups. One of the major findings from the East Flanders Prospective Twin Survey, where perinatal data such as chorion type and zygosity are established at birth, is that MZ twin pairs have a significantly higher risk of perinatal mortality than DZ twin pairs; however, the difference was limited to the MC subgroup and was especially the case before birth [14]. A large Danish study comprising 2043 twin pregnancies with ultrasound chorionicity determination before 15 weeks of gestation

confirmed a higher mortality rate for MC compared with DC twins, but this difference leveled off after 24 weeks of gestation and neonatal mortality was not correlated with chorionicity [33]. A study of 4191 OS twins and 10,875 SS twin pairs born in Sweden from 1973 to 1989 found that pregnancy loss was twice as high in SS compared with OS twins [34]. In accordance with these results, we found the largest difference in mortality between OS and SS twins for stillbirths.

Another reason for the better early-life survival for OS than SS boys may be lower exposure to prenatal testosterone. Transfer of testosterone is assumed based on animal studies which have demonstrated that any fetus, independent of sex, located between two male fetuses, has a higher concentration of testosterone than a fetus located between two females [12]. There are no studies of amniotic fluid testosterone levels from OS and SS twin pairs [35], and studies comparing OS with SS females have shown inconclusive results with the most consistent evidence to support the TTT hypothesis coming from studies of perception and cognition [11]. Several studies that have investigated co-twin effects in males have failed to identify differences between OS and SS male twins, for example, with regard to tooth size [36], academic performance [37], and cancer [38]. However, animal studies find that males exhibit reduced immune responses and increased intensity and prevalence of infections compared with females [4], and in humans, there is evidence that girl infants have lower mortality from infections [6] and respiratory conditions [7] compared with boys, which may reflect the immunosuppressive effects of testosterone [4,5]. Therefore, deaths with an infectious and respiratory disease underlying death cause are particularly interesting. We found that SS boys had higher mortality from infectious diseases compared with OS boys. Moreover, the better survival for boys with a girl co-twin persisted during the first year of life. These findings may provide evidence for the TTT hypothesis. Our results showing better early-life survival for OS than for SS boys were in line with the recent Swedish cohort study [17]. For girls, we did not find any significant differences between OS and SS twins in the hypothesized direction according to the TTT hypothesis, not either in the sensitivity analyses where the UZ twins were randomly distributed between the MZ and ssDZ twins. On the contrary, OS girls had significantly better survival than SS girls, although the mortality of OS girls was not significantly different from that of their twin brothers.

In agreement with previous studies [39,40], we found that the mortality risk has declined steadily for twins during the last decades. A study from Japan found that the decline in perinatal mortality of OS twins has been slower than that of SS twins since 1984 [41]. A study from the USA found that the perinatal mortality decreased for MZ twin pairs throughout the period 1980–2004, whereas the mortality for the DZ twin pairs decreased until the mid-1990s and increased slightly thereafter [42]. The authors suggest that advancing maternal age and increasing use of fertility treatments may be a reason for the stalling of the decline in perinatal mortality rates. Also, we observed no decrease in mortality for live-born OS twins between the 1990s and the 2000s.

The major strengths of this study are the availability of reliable mortality data from a large population-based register for all twins born 1973–2009 with complete follow-up. To our knowledge, this is the largest study of mortality risks among OS and SS twins. We were also able to analyze stillbirth and perinatal mortality for a large subgroup of OS and SS twins.



The important limitation of this study was the lack of data on twin zygosity and chorionicity. No information on zygosity was available for 51.4% of the live-born SS twins. OS twins are by definition DZ (i.e., all OS twins also those who dies shortly after birth are included in this group). If we exclude the UZ twins, the SS twin group consists exclusively of twin pairs in which both survived infancy and hereby could have their zygosity determined. Therefore, twins with known zygosity are not comparable with OS twins and bias will be present in the associations toward higher mortality for the OS twins. Therefore, it was not possible to evaluate whether ssDZ twins and OS twins have similar mortality, which is the most valid comparison of the TTT hypothesis.

In summary, this large study of Danish twins confirmed a higher mortality for twin boys than twin girls and found significantly lower perinatal mortality for OS than for SS twins of both sexes. This may at least partly be due to the inclusion of MZ twins with higher perinatal mortality in the SS twin groups. The results for OS girls were in the opposite direction as predicted by the TTT hypothesis, whereas the OS-SS comparison for boys regarding mortality and cause of death pattern provided some evidence for the TTT hypothesis. Future studies with the possibilities of including information on zygosity and/or chorionicity should investigate differences between OS and ssDZ twins only.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## Acknowledgments

This work was supported by the National Institute on Aging (NIA-PO1-AG08761, NIAP01-AG031719) and the European Union's Seventh Framework Program (FP7/2007–2011) under grant agreement number 259679.

K.C. holds a Chair at the Danish Institute for Advanced Study.

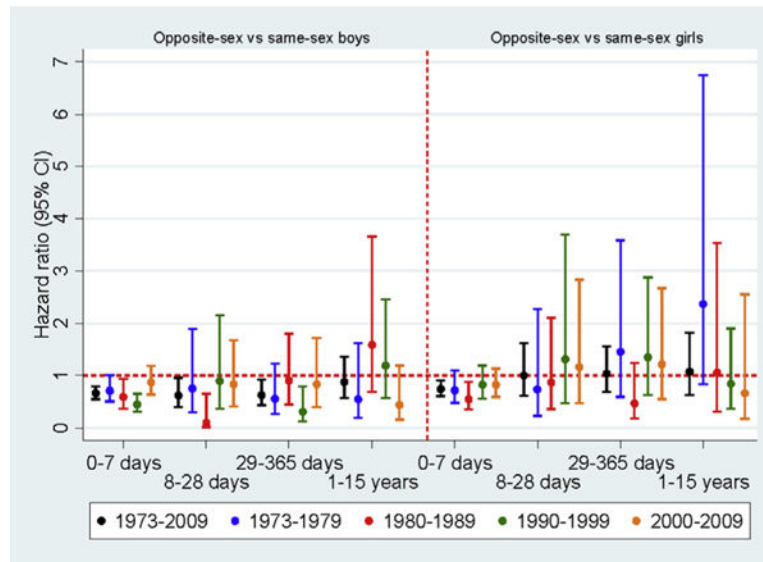
## References

1. Elsmén E, Steen M, Hellström-Westas L. Sex and gender differences in newborn infants: why are boys at increased risk? *J Mens Health Gend.* 2004; 1(4):303–11.
2. Sidebotham P, Fraser J, Covington T, Freemantle J, Petrou S, Pulikottil-Jacob R, et al. Understanding why children die in high-income countries. *Lancet.* 2014; 384(9946):915–27. [PubMed: 25209491]
3. Drevenstedt GL, Crimmins EM, Vasunilashorn S, Finch CE. The rise and fall of excess male infant mortality. *Proc Natl Acad Sci U S A.* 2008; 105(13):5016–21. [PubMed: 18362357]
4. Klein SL. Hormones and mating system affect sex and species differences in immune function among vertebrates. *Behav Processes.* 2000; 51(1–3):149–66. [PubMed: 11074318]
5. Trigunaite A, Dima J, Jørgensen TN. Suppressive effects of androgens on the immune system. *Cell Immunol.* 2015; 294(2):87–94. [PubMed: 25708485]
6. Person MK, Esposito DH, Holman RC, Mehal JM, Stoll BJ. Risk factors for infectious disease death among infants in the United States. *Pediatr Infect Dis J.* 2014; 33(11):e280–5. [PubMed: 24853540]
7. Mage DT, Donner EM. The fifty percent male excess of infant respiratory mortality. *Acta Paediatr.* 2004; 93(9):1210–5. [PubMed: 15384886]
8. McMillen MM. Differential mortality by sex in fetal and neonatal deaths. *Science.* 1979; 204(4388): 89–91. [PubMed: 571144]
9. Ryan BC, Vandenberg JG. Intrauterine position effects. *Neurosci Biobehav Rev.* 2002; 26(6):665–78. [PubMed: 12479841]

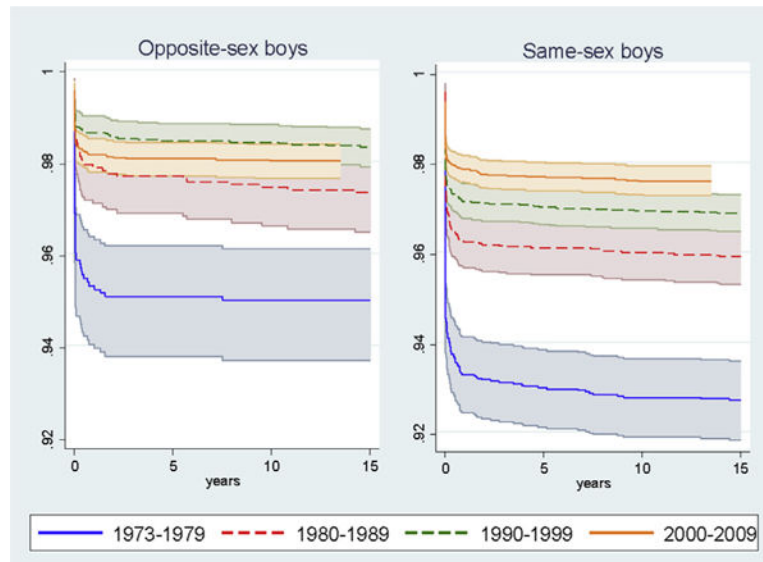


10. Even MD, vom Saal FS. Seminal vesicle and preputial gland response to steroids in adult male mice is influenced by prior intrauterine position. *Physiol Behav.* 1992; 51(1):11–6. [PubMed: 1741435]
11. Tapp AL, Maybery MT, Whitehouse AJO. Evaluating the twin testosterone transfer hypothesis: a review of the empirical evidence. *Horm Behav.* 2011; 60(5):713–22. [PubMed: 21893061]
12. Cohen-Bendahan CC, van de Beek C, Berenbaum SA. Prenatal sex hormone effects on child and adult sex-typed behavior: methods and findings. *Neurosci Biobehav Rev.* 2005; 29(2):353–84. [PubMed: 15811504]
13. Hall JG. Twinning. *Lancet.* 2003; 362(9385):735–43. [PubMed: 12957099]
14. Loos R, Derom C, Vlietinck R, Derom R. The East Flanders Prospective Twin Survey (Belgium): a population-based register. *Twin Res.* 1998; 1(4):167–75. [PubMed: 10100808]
15. Chen SJ, Vohr BR, Oh W. Effects of birth order, gender, and intrauterine growth retardation on the outcome of very low birth weight in twins. *J Pediatr.* 1993; 123(1):132–6. [PubMed: 8320607]
16. Powers WF, Wampler NS. Further defining the risks confronting twins. *Am J Obstet Gynecol.* 1996; 175(6):1522–8. [PubMed: 8987936]
17. Steen EE, Kallen K, Marsal K, Norman M, Hellstrom-Westas L. Impact of sex on perinatal mortality and morbidity in twins. *J Perinat Med.* 2014; 42(2):225–31. [PubMed: 24222256]
18. Shinwell ES, Reichman B, Lerner-Geva L, Boyko V, Blickstein I. “Masculinizing” effect on respiratory morbidity in girls from unlike-sex preterm twins: a possible transchorionic paracrine effect. *Pediatrics.* 2007; 120(3):e447–53. [PubMed: 17766488]
19. Aaby P, Molbak K. Siblings of opposite sex as a risk factor for child mortality. *BMJ.* 1990; 301(6744):143–5. [PubMed: 2390598]
20. Aaby P, Pison G, Desgrees du LA, Andersen M. Lower mortality for female-female twins than male-male and male-female twins in rural Senegal. *Epidemiology.* 1995; 6(4):419–22. [PubMed: 7548352]
21. Skytthe A, Ohm KK, Vilstrup HN, Christensen K. The Danish Twin Registry. *Scand J Public Health.* 2011; 39(7 Suppl):75–8. [PubMed: 21775358]
22. Skytthe A, Christiansen L, Kyvik KO, Bodker FL, Hvidberg L, Petersen I, et al. The Danish Twin Registry: linking surveys, national registers, and biological information. *Twin Res Hum Genet.* 2013; 16(1):104–11. [PubMed: 23084092]
23. Christiansen L, Frederiksen H, Schousboe K, Skytthe A, von Wurmb-Schwark N, Christensen K, et al. Age- and sex-differences in the validity of questionnaire-based zygosity in twins. *Twin Res.* 2003; 6(4):275–8. [PubMed: 14511432]
24. Skytthe A, Kyvik K, Holm NV, Vaupel JW, Christensen K. The Danish Twin Registry: 127 birth cohorts of twins. *Twin Res.* 2002; 5(5):352–7. [PubMed: 12537858]
25. Pedersen CB. The Danish Civil Registration System. *Scand J Public Health.* 2011; 39(7 Suppl):22–5. [PubMed: 21775345]
26. Helweg-Larsen K. The Danish Register of Causes of Death. *Scand J Public Health.* 2011; 39(7 Suppl):26–9.
27. Jensen VM, Rasmussen AW. Danish education registers. *Scand J Public Health.* 2011; 39(7 Suppl): 91–4. [PubMed: 21775362]
28. Knudsen LB, Olsen J. The Danish Medical Birth Registry. *Dan Med Bull.* 1998; 45(3):320–3. [PubMed: 9675544]
29. United National Educational Scientific and Cultural Organisation, International Standard Classification of Education (ISCED) 1997. 2006. Available at: <http://www.uis.unesco.org/Library/Documents/isced97-en.pdf>
30. Wilcox, AJ. Stillbirth and infant mortality Fertility and pregnancy an epidemiologic perspective. New York: Oxford University Press; 2010. p. 164–82.
31. Lin DY, Wei LJ. The robust inference for the Cox proportional hazards model. *J Am Stat Assoc.* 1989; 84(408):1074–8.
32. Pongou R. Why is infant mortality higher in boys than in girls? A new hypothesis based on preconception environment and evidence from a large sample of twins. *Demography.* 2013; 50(2): 421–44. [PubMed: 23151996]

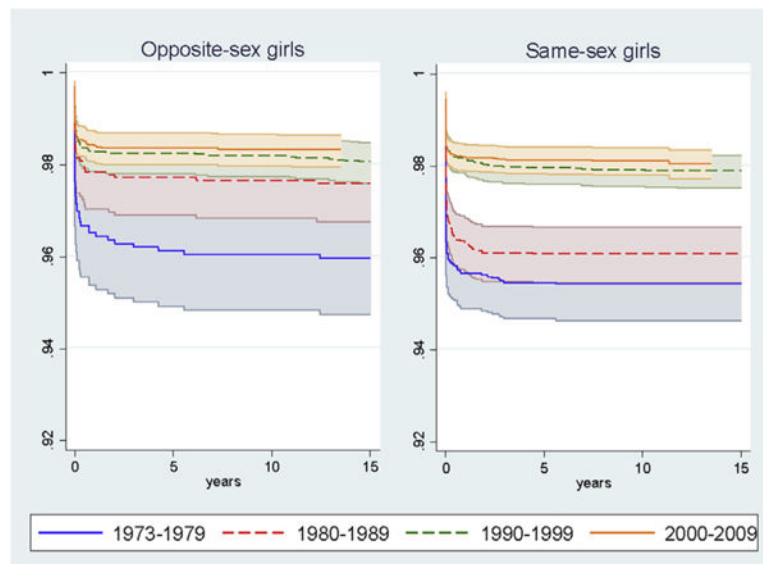
33. Oldenburg A, Rode L, Bodker B, Ersbak V, Holmskov A, Jorgensen FS, et al. Influence of chorionicity on perinatal outcome in a large cohort of Danish twin pregnancies. *Ultrasound Obstet Gynecol.* 2012; 39(1):69–74. [PubMed: 21830245]
34. Rydhstrom H. Discordant birthweight and late fetal death in like-sexed and unlike-sexed twin pairs: a population-based study. *Br J Obstet Gynaecol.* 1994; 101(9):765–9. [PubMed: 7947526]
35. Korsoff P, Bogl LH, Korhonen P, Kangas AJ, Soininen P, Ala-Korpela M, et al. A comparison of anthropometric, metabolic, and reproductive characteristics of young adult women from opposite-sex and same-sex twin pairs. *Front Endocrinol (Lausanne).* 2014; 5:28. [PubMed: 24639667]
36. Dempsey PJ, Townsend GC, Richards LC. Increased tooth crown size in females with twin brothers: evidence for hormonal diffusion between human twins in utero. *Am J Hum Biol.* 1999; 11(5):577–86. [PubMed: 11533976]
37. Ahrenfeldt L, Petersen I, Johnson W, Christensen K. Academic performance of opposite-sex and same-sex twins in adolescence: a Danish national cohort study. *Horm Behav.* 2015; 69:123–31. [PubMed: 25655669]
38. Ahrenfeldt LJ, Skytthe A, Moller S, Czene K, Adami HO, Mucci LA, et al. Risk of sex-specific cancers in opposite-sex and same-sex twins in Denmark and Sweden. *Cancer Epidemiol Biomarkers Prev.* 2015; 24:1622–8. [PubMed: 26282631]
39. Luke B, Brown MB. The changing risk of infant mortality by gestation, plurality, and race: 1989–1991 versus 1999–2001. *Pediatrics.* 2006; 118(6):2488–97. [PubMed: 17142535]
40. Oestergaard MZ, Inoue M, Yoshida S, Mahanani WR, Gore FM, Cousens S, et al. Neonatal mortality levels for 193 countries in 2009 with trends since 1990: a systematic analysis of progress, projections, and priorities. *PLoS Med.* 2011; 8(8):e1001080. [PubMed: 21918640]
41. Kato N. Recent decelerated decline in perinatal mortality rate of unlike-sexed twins in Japan. *Paediatr Perinat Epidemiol.* 2004; 18(3):192–5. [PubMed: 15130158]
42. Hartley RS, Hitti J. Increasing rates of sex-discordant twins no longer correspond to decreasing perinatal mortality rates. *J Perinat Med.* 2008; 36(3):228–34. [PubMed: 18576932]



**Fig. 1.** Hazard ratios and 95% confidence intervals (CIs) for all-cause mortality 0–15 years stratified on age and decades for live-born opposite-sex and same-sex twins born in Denmark during 1973–2009.



**Fig. 2.** Kaplan-Meier curves with 95% confidence bands for all-cause mortality 0–15 years for the last 4 decades for opposite-sex and same-sex live-born twin boys in Denmark during 1973–2009.



**Fig. 3.** Kaplan-Meier curves with 95% confidence bands for all-cause mortality 0–15 years for the last 4 decades for opposite-sex and same-sex live-born twin girls in Denmark during 1973–2009.

Table 1

Characteristics of live-born twins in Denmark during 1973–2009

Characteristics	Twin boys		Twin girls		All	
	Opposite sex	Same sex	All	Opposite sex	Same sex	All
Study population <i>N</i>	12,051	22,901	34,952	12,033	21,644	33,677
Deaths before age 15* (% of study population <i>N</i> )	271 (2.25)	825 (3.60)	1096 (3.14)	252 (2.09)	585 (2.70)	837 (2.49)
Early neonatal deaths (0–7 d)	179 (1.49)	551 (2.41)	730 (2.09)	166 (1.38)	424 (1.96)	590 (1.75)
Late neonatal deaths (8–28 d)	27 (0.22)	88 (0.38)	115 (0.33)	27 (0.22)	53 (0.24)	80 (0.24)
Postneonatal deaths (29–365 d)	35 (0.29)	117 (0.51)	152 (0.43)	36 (0.30)	67 (0.31)	103 (0.31)
Child mortality (1–15 y)	30 (0.25)	69 (0.30)	99 (0.28)	23 (0.19)	41 (0.19)	64 (0.19)
Emigrated*	187 (1.6)	346 (1.5)	533 (1.5)	185 (1.5)	363 (1.7)	548 (1.6)
Mean (SD) birth weight, <sup>†</sup> <i>N</i>	2581 (603), 1913	2484 (630), 22,406	2518 (623), 34,319	2470 (584), 11,909	2416 (593), 21,178	2435 (591), 33,087
Mean (SD) maternal age <sup>‡</sup>	31.05 (4.6)	30.04 (4.8)	30.39 (4.8)	31.05 (4.6)	30.00 (4.8)	30.37 (4.8)
–20	69 (0.6)	334 (1.5)	403 (1.2)	69 (0.6)	343 (1.6)	412 (1.2)
20–34	9561 (79.4)	18,965 (82.9)	28,526 (81.7)	9551 (79.4)	17,865 (82.6)	27,416 (81.5)
35+	2415 (20.1)	3591 (15.7)	6006 (17.2)	2407 (20.0)	3419 (15.8)	5826 (17.3)
Maternal education <sup>§</sup>						
Primary and lower secondary	2231 (18.8)	4609 (20.4)	6840 (19.9)	2226 (18.8)	4412 (20.8)	6638 (20.0)
Upper and post-secondary	4852 (40.8)	9272 (41.1)	14,124 (41.0)	4847 (40.9)	8702 (40.9)	13,549 (40.9)
Tertiary	4798 (40.4)	8694 (38.5)	13,492 (39.2)	4790 (40.4)	8153 (38.3)	12,943 (39.1)

Values are numbers (percentages) unless otherwise stated.

\* Before 15 years or before July 1, 2013.

<sup>†</sup> In grams.<sup>‡</sup> In years.<sup>§</sup> Highest completed education during 1981–2012. Primary and lower secondary education refers to less than 10 years of education, upper and post-secondary education refers to 10–12 years education, and tertiary education refers to more than 12 years of education.

**Table 2**

Hazard ratios (HRs) and 95% confidence intervals (CIs) for all-cause mortality stratified on age and adjusted for decade for live-born boy versus girl twins and for opposite-sex versus same-sex twins born in Denmark during 1973 to 2009

<b>1973–2009</b>	<b>0–7 d</b>	<b>8–28 d</b>	<b>29–365 d</b>	<b>1–15 y</b>
	<b>HR (95% CI)</b>	<b>HR (95% CI)</b>	<b>HR (95% CI)</b>	<b>HR (95% CI)</b>
Boys versus girls				
All twins	1.19 (1.05–1.34)	1.38 (1.03–1.86)	1.42 (1.10–1.83)	1.49 (1.09–2.04)
Within opposite-sex twin pairs	1.06 (0.92–1.22)	1.00 (0.61–1.66)	0.97 (0.62–1.54)	1.31 (0.78–2.20)
Opposite-sex versus same-sex boys	0.66 (0.55–0.79)	0.62 (0.40–0.96)	0.63 (0.43–0.92)	0.88 (0.57–1.36)
Adjusted for maternal age and education	0.66 (0.55–0.79)	0.63 (0.40–0.97)	0.61 (0.42–0.90)	0.91 (0.59–1.41)
Opposite-sex versus same-sex girls	0.74 (0.61–0.90)	1.00 (0.62–1.61)	1.04 (0.69–1.56)	1.07 (0.63–1.82)
Adjusted for maternal age and education	0.76 (0.63–0.93)	0.99 (0.61–1.60)	1.09 (0.73–1.65)	1.07 (0.63–1.84)



**Table 3**

Relative risks (RRs) and 95% confidence intervals (CIs) for stillbirths and perinatal mortality adjusted for decades among girl and boy twins and opposite-sex and same-sex twins born in Denmark during 1973 to 1996

1973–1996	Stillbirths	Perinatal mortality <sup>*</sup>
	RR (95% CI)	RR (95% CI)
Boys versus girls		
All twins	1.24 (1.03–1.49)	1.22 (1.05–1.42)
Within opposite-sex twin pairs	1.41 (0.94–2.12)	1.01 (0.83–1.24)
Opposite-sex versus same-sex boys	0.48 (0.36–0.65)	0.73 (0.57–0.94)
Opposite-sex versus same-sex girls	0.42 (0.29–0.59)	0.58 (0.46–0.74)

<sup>\*</sup> Stillbirths and mortality among live births within the first 7 days of life.